The wax and wane of Phaeocystis globosa blooms

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Chapter 3

The development of a *Phaeocystis* bloom in a mesocosm experiment in relation to nutrients, irradiance and coexisting algae

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"Il y a donc là un point à élucider"

G. Hamel (1930)

ABSTRACT. A widespread hypothesis is that man-induced eutrophication, an increase in nitrogen and/or phosphorus rather than silicon, gives a competitive advantage to the non-silicon using flagellates against the silicon dependent diatoms. Some authors suggest that such a mechanism could explain the intensification of the *Phaeocystis* blooms observed in the last decades in the coastal zones of the North Sea.
A mesocosm experiment aiming to investigate this mechanism was performed in two 3 m^3 mesocosms. Light climate, mixing regime and nutrient concentrations reproduced conditions in Dutch coastal waters. Silicon was added to ensure a phytoplankton development without Si limitation. Massive blooms of the colony forming prymnesiophyte *Phaeocystis globosa* developed in both mesocosms and outcompeted the diatom community. Nutrients were continuously added and no nutrient limitation occurred during the bloom development. After four weeks, the *Phaeocystis* bloom collapsed and diatoms reappeared in both tanks. The dominance of *Phaeocystis* in a non-silicon-limited environment constitutes an original observation which is in contradiction with the conclusions of some previously published works.

Introduction

Irradiance and nutrients (nitrogen (N), phosphorus (P), silicon (Si)) are essential factors controlling primary production, phytoplankton biomass and species succession. Irradiance determines the season of primary production at temperate latitudes. Depending on the nutrient concentrations, irradiance can also become a limiting factor for primary production when light attenuation is increased by suspended sediment and high phytoplankton concentrations (self-shading). If irradiance is not limiting, the quantity of nutrients available for primary producers determines the potential phytoplankton biomass.

The actual phytoplankton biomass is also controlled by loss factors such as sedimentation, autolysis and grazing. There are strong indications that sedimentation and autolysis are closely coupled with the physiological properties of the cell. These properties are often related to the availability of the controlling factor (nutrient or light) (van Boekel *et al.* 1992, Waite *et al.* 1992b). Moreover, there seems to be a positive correlation between the cell size and the intrinsic sinking rate (Smayda 1970) whereas a negative correlation has been found between the cell size and its edibility by microzooplankton (Verity 1986, Burkill *et al.* 1987).

Riegman *et al.* (1993a) proposed a food web model, combining the import rate of the controlling factor for the primary production (nutrient or light), the sedimentation and the grazing rates to describe the phytoplankton community structure in response to eutrophication. At a low import rate of the controlling factor, the growth rate of large phytoplankton species cannot exceed their high sedimentation rate. Consequently, the phytoplankton community is dominated by small species. When there is a high import rate of the controlling factor, the

biomass of the small phytoplankton species is rapidly limited by the grazing of microzooplankton, whereas the large phytoplankton species escape grazing and could compensate their sedimentation rate in these favourable growth conditions. This functional shift can be interpreted as a response of the system allowing an optimal utilization of the elevated import of the controlling factor (nutrient or light), a crucial step in the eutrophication process.

Eutrophication in coastal and stratified areas has led to the development of massive algal blooms, hypoxia and shifts in phytoplankton species composition in many areas in the world (Smayda 1990, Vollenweider *et al.* 1992). In Chesapeake Bay increasing levels of eutrophication over the last decades led to greater abundance's and extended periods of phytoplankton growth (Marshall and Lacouture 1986). Man induced eutrophication usually corresponds with an increase of N and P rather than Si and may therefore favour non Si dependent algae against diatoms (Officer and Ryther 1980). In the inner German Bight flagellate biomass increased 10- to 15-fold while diatom biomass slightly decreased between 1962 and 1984 (Radach and Berg 1986). The authors related this to a sharp increase of the N:Si ratio because the nitrate concentration increased nearly fourfold whereas silicate dropped to one fifth during the same period.

In Dutch coastal waters, the increase in phytoplankton biomass and shifts in its species composition (Cadée and Hegeman 1986, Radach *et al.* 1990) have also been attributed to eutrophication. Cadée and Hegeman (1986) reported an increase of both the *Phaeocystis* spring bloom cell numbers (two-fold) and the duration of the spring bloom (eight-fold) in the Marsdiep over the period 1973-1985. Riegman (1991) found that non-colonial *Phaeocystis* became dominant in N-limited chemostats. In P-limited chemostats, *Phaeocystis* was outcompeted. In discontinuously diluted batch cultures, *Phaeocystis* colony formation only occurred when NO_3^- was the sole limiting N source, under P limitation ($P < 0.23$ μM) no colonies were formed. Although these observations seem contradictory to previous findings (Veldhuis and Admiraal 1987, Veldhuis *et al.* 1991) where colony formation was observed at P concentrations below 0.2 μM (and thus limiting with a K_s for growth estimated as 0.7 μM), Riegman (1991) concluded that the summer colonial bloom of *Phaeocystis* in the Marsdiep was caused by major shifts in the N:P ratio (from P to N dominated limitation) and in the NH_4^+:NO_3^- ratios which occurred since the year 1978. However, colonial *Phaeocystis* summer blooms were observed before 1978 (Cadée and Hegeman 1986) and the ratio shifted due to an unequal increase of both P and N discharge (Riegman *et al.* 1992). It can be argued that, as simultaneous increases of N and P loads occurred, the high loads themselves could have caused an increased duration of the *Phaeocystis* bloom (Peperzak 1993).

The question remains why it is, amongst all the non-diatom species, that *Phaeocystis* profits most from elevated nutrient availability. Veldhuis *et al.* (1991) observed that the ability of *Phaeocystis* to switch from flagellate to colonial forms with completely different requirements broadens its ecological niche and

could confer a competitive advantage in transient state nutrient conditions to this species. *Phaeocystis* colonial cells are able to perform protein synthesis under light limitation at the expense of the extra-cellular muco-polysaccharides synthesised in the light (Lancelot and Mathot 1985). This feature could be advantageous for *Phaeocystis* colonial cells, especially by transient light conditions such as in turbid coastal areas. *Phaeocystis* colonies have been shown to be less edible to microzooplankton and some mesozooplankton species than smaller cells (Verity and Smayda 1989). This could also be one of the features contributing to the *Phaeocystis* dominance during the summer blooms.

The non-toxic *Phaeocystis* is regarded as a nuisance alga mainly because of the intensive foam production during the wane of the bloom. In the Southern Bight of the North Sea the *Phaeocystis* bloom consists of a high spring peak appearing usually some weeks after the spring diatom peak (Gieskes and Kraay 1975, Cadée and Hegeman 1986).

Several hypotheses have been proposed to explain the diatom/*Phaeocystis* succession. According to Egge and Aksnes (1992), a high inherent growth rate of diatoms at non-limiting Si concentrations may prevent *Phaeocystis* to break through at high Si concentrations. Under N or P limiting circumstances, diatoms would have a competitive advantage over *Phaeocystis* colonies because they have a higher storage capacity for these nutrients (Jahnke 1989). *Phaeocystis* blooms could develop utilising the remaining N and P after the diatom spring bloom becomes Si limited (van Bennekom *et al.* 1975).

The fact that the spring *Phaeocystis* bloom occurs after the diatom bloom may also result from specific requirements for other environmental factors such as light and/or temperature. The *Phaeocystis* bloom may need higher temperature and irradiance than diatoms (Veldhuis *et al.* 1986). Under controlled experimental conditions, *Phaeocystis* was unable to develop colonies when daily irradiance was less than 100 W h m^{-2} day^{-1} (Peperzak 1993).

This mesocosm experiment was used to test whether diatoms could systematically dominate phytoplankton assemblages under silicon-replete conditions as stated by Officer and Ryther (1980) and Egge and Aksnes (1992).

Methods

experimental scheme

The experiment was executed at the National Institute for Coastal and Marine Management (RIKZ) fieldstation 'Jacobahaven' situated at the mouth of the Oosterschelde (S.W. Netherlands). The experiment started on 01-06-1992 and lasted 40 days. Two 3000 l mesocosms (mesocosm 1 and 2) were filled with sea water originating from the Oosterschelde, filtered over 100 µm gauze and complemented with 100 l non-filtered sea water. The purpose of the 100 µm

filtration was to avoid the introduction of large predators which could have significantly and selectively influence the phytoplankton development, and to favour the reproducibility between the mesocosms (possible patchy distribution of large organisms). As a consequence, some large diatoms and *Phaeocystis* colonies were discarded. Nevertheless 12 diatom species were present in the mesocosms with among them, large chain forming species as *Rhizosolenia shrubsolei*, *Eucampia zodiacus* and *Odontella aurita*.

Rotating mixers mixed the mesocosms water continuously. Continuously operating scrapers limited the fouling on the mesocosm walls. Optical diffusers above the mesocosms ensured a homogeneous light climate in the mesocosms. The mixing and the light climate in the mesocosms reproduced (on scale) the conditions of a 10 m water column in the Dutch coastal zone. N ($NaNO_3$), P (NaH_2PO_4) and Si ($Na_2SiO_3.5H_2O$) were added to establish initial nutrient concentrations (N = 95 µM, P = 3 µM, Si = 20 µM) sufficient to promote a massive phytoplankton development. The Si concentrations were adjusted relatively to the N and P concentrations to prevent any Si limitation for the diatoms. Nutrients were periodically added in order to compensate for phytoplankton uptake (Figure 3.3). On average, the daily nutrient inputs were 3.71 µM N, 0.23 µM P and 1.52 µM Si for mesocosm 1 and 3.56 µM N, 0.18 µM P and 1.40 µM Si for mesocosm 2. Vitamins (0.01 nM of thiamine, biotin, B12) and trace metals (10 nM Fe, 1 nM Mn, 0.1 nM of Zn, Co, Mn, Mo) were added together with the macro-nutrients.

sampling and analytic procedures

Daily integral irradiance (PAR in W h m^{-2} over one day) was recorded with a Kipp and Zonen Solar Integrator in combination with a light sensor (developed by Wageningen Agricultural University) mounted on top of a nearby building. To account for light absorption by the optical diffuser above the mesocosms the measured daily integral irradiance was multiplied by 0.7. Irradiance attenuation in the water column was measured in duplicate 3 times a week with a LiCor underwater quantum spherical sensor (SPA-QUANTUM). The apparent attenuation coefficient (K_d in m^{-1}) was calculated using linear regression (equation 3.1):

$$\ln (I_z / I_0) = -K_d * z \qquad [3.1]$$

with I_0: incident irradiance at surface and I_z: incident irradiance at z meters (W m^{-2}).

From daily surface irradiance (W h m^{-2} day^{-1}) and K_d (m^{-1}), the daily irradiance averaged over the water column follows from equation 3.2:

$$DI = DI_0 * (1 - e^{-K_d * z}) / (K_d * z) \qquad [3.2]$$

with DI_0 is daily irradiance at surface (W h m^{-2} day^{-1}) and DI is daily irradiance averaged over the water column (W h m^{-2} day^{-1}) and z (m) is the depth of the water column.

Water samples were collected 3 times a week. Dissolved inorganic nutrients (N as $NH_4^+ + NO_3^- + NO_2^-$), P as PO_4^{3-} and Si as $Si(OH)_4$ were determined with an autoanalyzer according to Grasshoff *et al.* (1983). The detection limits were: 0.14 µM $N\text{-}NO_3^-$, 0.07 µM $N\text{-}NO_2^-$, 0.29 µM $N\text{-}NH_4^+$, 0.06 µM $P\text{-}PO_4^{3-}$, 0.36 µM Si-$Si(OH)_4$. Chlorophyll *a* and phaeopigments were extracted on GF/C filters according to Gieskes and Kraay (1984) and analysed by HPLC with a 85-100% acetone/water-water gradient, using a reversed phase RP18 Novopack column (Waters) in a Spectra Physics Chromatography station. Chlorophyll *a* was detected with a Perkin Elmer LS-2B fluorometer (excitation: 410-430 nm; emission: > 530 nm). A standard chlorophyll *a* solution was used for calibration.

Primary production was measured by incubating water samples for 2 hours with 185 kBq ^{14}C-bicarbonate (Amersham) at irradiances of 0, 2.3, 5.3, 11.7, 29.6, 61.1, 144.3 and 332.3 W m^{-2} in a temperature-controlled incubator. Samples were processed according to Peeters *et al.* (1991a). Irradiance (I, W m^{-2}) and production (P, mg C mg Chla^{-1} h^{-1}) were fitted to the model (Eilers and Peeters 1988):

$$P(I) = I / (a\ I^2 + b\ I + c) \qquad [3.3]$$

Maximum production (P_{max}), irradiance half saturation constant (I_k) and daily integrated production were calculated combining irradiance, chlorophyll *a* concentration and P/I curve parameters using PRODUK software (Duin and Bil 1988).

Indications over the degree of light limitation were obtained from the P to P_{max} ratio. The following scale was used to interpret the $P:P_{max}$ ratio. $P:P_{max}$ between 0.5 and 0.1: moderate limitation, $P:P_{max}$ below 0.1: extreme limitation (Peeters *et al.* 1993). Care has, however, to be taken with the interpretation of this ratio as it considers the gross production. The light limitation for the net growth (µ) could be more intense than suggested by the $P:P_{max}$ ratio since even if algae grow at μ_{max}, the corresponding P is lower than the P_{max}. The $P:P_{max}$ is calculated as follows:

$$P / P_{max} = I_{av} / (I_k + I_{av}) \qquad [3.4]$$

Where I_{av} is the daily averaged irradiance (W m^{-2}) in the water column.

Deviations of the ratio P:N:Si = 1:16:16 (Gillbricht 1988) for dissolved nutrients were used as indicator for the first potentially limiting nutrient (Peeters *et al.* 1993). The concentration of this nutrient was then compared to the range of half-saturation constants for nutrient uptake: N = 1-2 µM; P = 0.1-0.5 µM; Si = 1-5 µM (Fisher *et al.* 1988, Peeters and Peperzak 1990).

Phytoplankton samples were fixed with acid Lugol's iodine solution. Phytoplankton cell numbers and species composition were determined by the

Utermöhl technique (Utermöhl 1958). Counts were made in 5 ml concentrated samples (x10) on an inverted microscope (magnification x160 and x400). Dense samples were counted in a hemocytometer.

Results

Due to variable weather conditions the surface irradiance changed erratically during the experiment (Figure 3.1a). The apparent light attenuation coefficient (K_d) increased from day 5 onwards and reached a value of 3 m^{-1} on day 17 in both mesocosms (Figure 3.1b). Then K_d decreased until days 31 to 33. An increase of K_d occurred in both mesocosms from day 33 onwards, followed by a new decrease in mesocosm 1 but not in mesocosm 2. Mean water column daily irradiance was mostly between 100 and 400 W h m^{-2} day^{-1} (Figure 3.1c). Mean water column daily irradiance at Noordwijk 10 for the same period in 1988-1990 was between 100 and 260 W h m^{-2} day^{-1} (Peeters *et al.* 1993). Light limitation coefficients (Figure 3.2) lay between 0.1 and 0.5, indicating moderate light limitation (Peeters *et al.* 1993). Increase of the light limitation observed around days 20 and 30 corresponded to decrease of the surface irradiance (Figure 3.1c).

The nutrient concentrations varied over time (Figure 3.3). Inputs until day 10 maintained (N) or increased (P, Si) nutrient concentrations. From day 10 onward the nutrient concentrations decreased in both mesocosms in spite of inputs on day 13 (N and P). Inputs of N and P on days 18 and 25 provoked marked concentration increases except for P in mesocosm 1. Since Si was not added after day 9, concentrations decreased in both mesocosms. The Si decrease was more pronounced in mesocosm 1, indicating higher biological uptake in mesocosm 1 compared to mesocosm 2.

P concentrations showed a similar difference. The N:P ratio remained at values higher than 16 during the whole experiment (Figure 3.4) indicating P as potentially limiting nutrient. However, minimum P concentrations were higher than 0.5 µM and therefore probably not actually limiting. The Si limitation programmed for the second half of the first experiment was not effectively established.

Chlorophyll *a* concentrations evolved in a comparable way in both mesocosms (Figure 3.5). From day 5 till day 18, concentrations increased in both mesocosms and reached values between 50 and 60 µg l^{-1}. From day 20 onwards there was a further increase in mesocosm 1 followed somewhat by mesocosm 2.

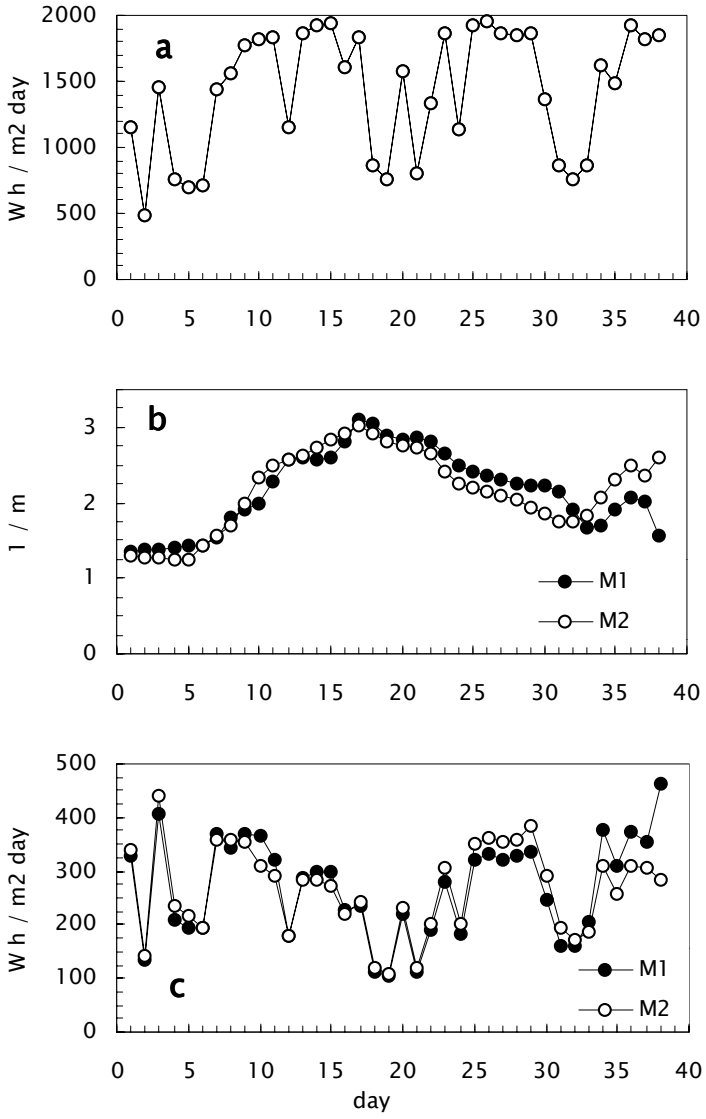

Figure 3.1. Surface daily irradiance (**a**), light attenuation coefficient (**b**) and mean water column daily irradiance (**c**) in mesocosm 1 (M1) and mesocosm 2 (M2).

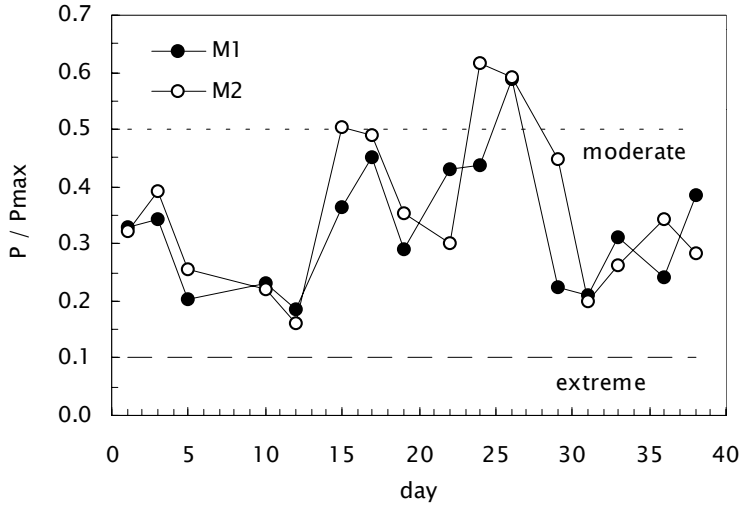

Figure 3.2. Light limitation coefficient ($P:P_{max}$) in mesocosm 1 (M1) and mesocosm 2 (M2).

Maximum chlorophyll *a* concentrations were reached in both mesocosms on day 24. From then on concentrations decreased until day 36. During the last days of the experiment concentrations increased again in mesocosm 2 but remained unchanged in mesocosm 1.

Daily primary production increased exponentially starting on day 5 till day 15, from 0.5 to almost 6 g C m^{-2} day^{-1} (Figure 3.6). Between day 15 and 17 daily primary production decreased to 2 g C m^{-2} day^{-1}. From day 19 to 26 the production in both mesocosms remained fairly constant. Between day 26 and 31 the daily primary production decreased continuously.

19 phytoplankton species and groups of species were identified (Table 3.1). Most of the time the prymnesiophyte *Phaeocystis* was numerically dominant. The diatom phytoplankton was dominated by two species: *Pseudo-nitzschia delicatissima* and *Skeletonema costatum*. The rest of the phytoplankton community mainly consisted of dinoflagellates, microflagellates and some diatoms which appeared episodically in the counts.

After one week diatoms started to grow together with *Phaeocystis* in both mesocosms (Figure 3.7). The diatoms reached their maximal density almost one week later and then declined in both mesocosms despite high nutrient concentrations available at that time (Figure 3.3). Colonies of *Phaeocystis* could be observed in both mesocosms.

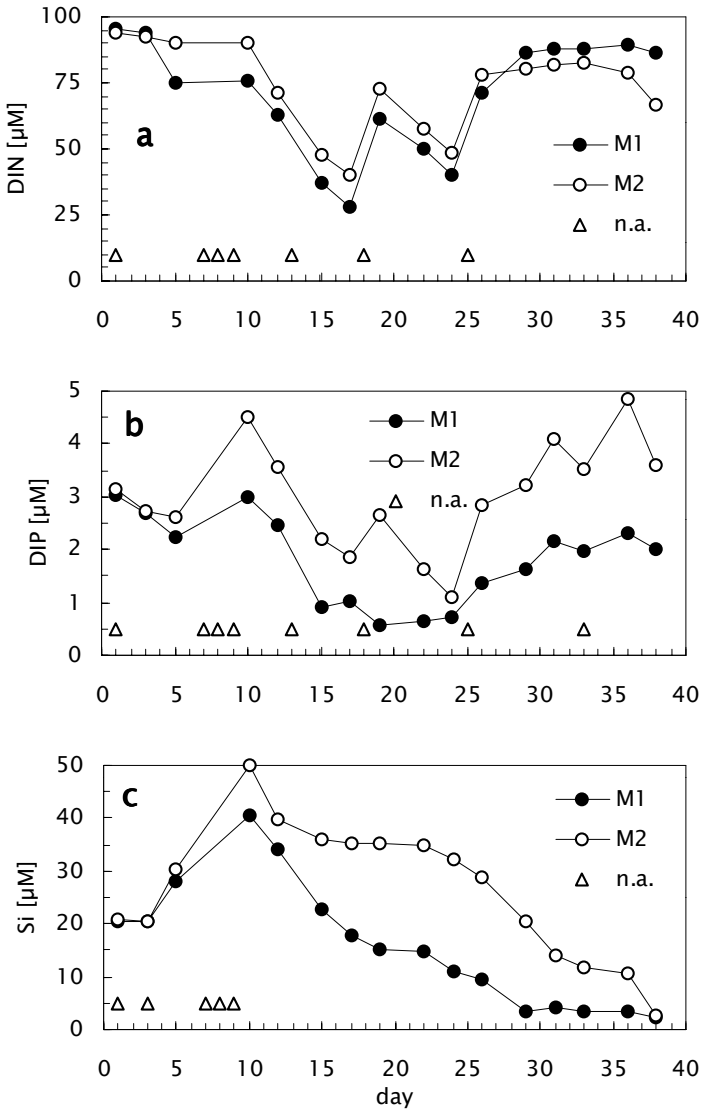

Figure 3.3. DIN (**a**), DIP (**b**) and Si (**c**) concentrations in mesocosm 1 (M1) and mesocosm 2 (M2). n.a. = nutrient addition

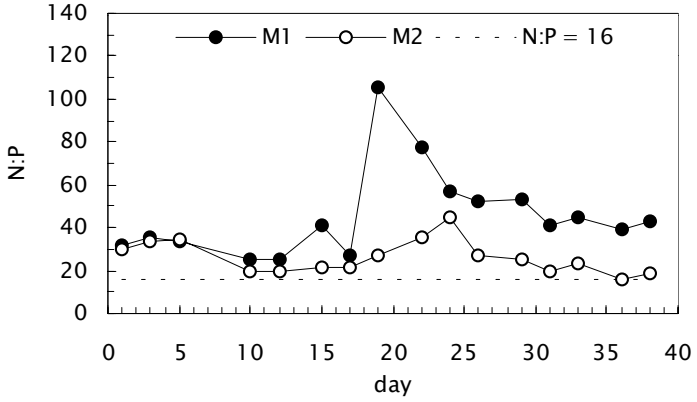

Figure 3.4. N:P ratios in mesocosm 1 (M1) and mesocosm 2 (M2).

The *Phaeocystis* population continued to grow and reached densities of almost 200 x 10^6 cells l^{-1} in both mesocosms between day 19 and 24. By this time primary production had declined (Figure 3.6) and the bloom collapsed within one week in both mesocosms. From day 26 onward, during the *Phaeocystis* decline, diatoms (mainly *S. costatum*) again developed in both mesocosms. In mesocosm 2 this development went on until the end of the experiment. In mesocosm 1 diatom development stopped after one week. In
the week following the decline of the *Phaeocystis* bloom, so called 'ghost colonies' were observed in both mesocosms. They consisted of colony residue (empty matrix) and disrupted envelopes still containing a few cells. From day 26 onwards microflagellates identified as *Phaeocystis* (cell diameter 3 to 4 µm) appeared in mesocosm 1 and to a lesser extent in mesocosm 2. Their numbers increased until the end of the experiment (Figure 3.8).

Discussion

The mesocosms were originally designed to reproduce the environmental conditions of monitoring station NW10 in the Dutch coastal zone. The nutrient concentrations measured at NW10 for the same period of year as the experiment (June/July) and averaged over the years 1980 to 1987 were: N = 62 µM, P = 1.16 µM and Si = 6.6 µM (Dutch monitoring programme of water quality). For comparison the mean concentrations measured during the present experiment were: N = 72 µM, P = 2.40 and Si = 21.1 µM. Except for Si, set at purpose to excessively high concentrations, the mean concentrations of the other macro-

Table 3.1. List of phytoplankton species growing in the mesocosm tanks during the experiment (ranked following decreasing numerical importance)

Phaeocystis sp.	dinoflagellates (unidentified)
microflagellates	*Protoperidinium bipes*
cryptophyceae	*Navicula* sp.
Skeletonema costatum	*Licmophora* sp.
Pseudo-nitzschia delicatissima	*Pseudo-nitzschia seriata*
Oxyrrhis marina	*Odontella aurita*
Amphora sp.	*Thalassiosira nordenskioldii*
pennate diatoms (unidentified)	*Rhizosolenia shrubsolei*
centric diatoms (unidentified)	*Eucampia zodiacus*
Nitzschia longissima	

nutrients did not differ much from field concentrations for the same period of the year.

The temperature fluctuated between 16 and 20°C during the experiment. For the same period of the year the temperature at NW10 was lower with a mean value (1980-1987) of 15.6°C.

Mixing is a very important factor as it determines how fast phytoplankton items will travel across the light gradient. The intensity of the currents generating the turbulent mixing will determine whether algal cells will settle or be stirred up from the bottom of the mesocosms. The mixing of the mesocosm water was set to reproduce the mixing time at NW10 in the first 10 m of the water column (45 min). For this purpose the rotation was set at 5 rpm corresponding to a tangential speed at the end of the mixer equalling 0.23 m s^{-1}. The current speed generated by the mixers were certainly lower than this value. For comparison, the averaged tidal currents measured at our reference station equal 0.4 m s^{-1}. Whereas the mixing time in the mesocosms was representative for the field, the current speed appears lower than at sea; this could have affected the sedimentation process in the mesocosms.

The phytoplankton species found in this experiment are common Oosterschelde and North Sea species (Gieskes and Kraay 1975, Bakker *et al.* 1990, Reid *et al.* 1990). Species such as *Nitzschia longissima*, *Amphora* sp., *Navicula* sp. and other unidentified pennate diatoms are typical fouling or phytobenthic algae. They probably originated from the mesocosm walls and were brought in suspension by the anti-fouling device and the mixer.

The collapse of the diatom bloom occurred without indications of any light or nutrient limitation. The reappearance of diatoms after the collapse of the *Phaeocystis* colonial bloom did not coincide with remarkable improvement of nutrient and light conditions in the mesocosms. Note that diatom cell numbers decreased as *Phaeocystis* cell numbers increased to bloom intensity, and that

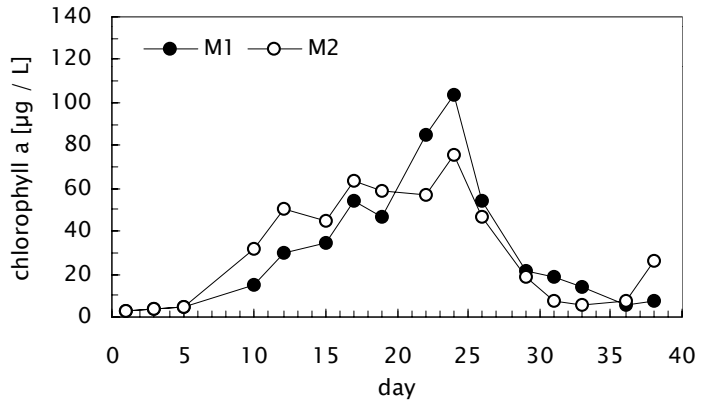

Figure 3.5. Chlorophyll *a* concentrations in mesocosm 1 (M1) and mesocosm 2 (M2).

diatoms increased again after the *Phaeocystis* colonies had disappeared. The following questions are raised: why were diatoms outcompeted by *Phaeocystis*, despite the presence of sufficiently high Si concentrations? Why did the *Phaeocystis* colonial bloom collapse? Several explanations can be proposed:

differential sedimentation rates

According to Smayda (1970) most of the non motile marine phytoplankton species sink. Bienfang (1982) observed that *Chaetoceros socialis* colonies had a very low settling rate (less than 0.40 m day^{-1}) when compared with the settling rate of other diatoms (≈ 1.39 m day^{-1} for *Leptocylindricus danicus* and *Thalassiosira decipiens*). *C. socialis* are spheres of several millimetre in diameter consisting of coiled chains of many hundred cells. Bienfang (1982) attributed the low settling rate to the contribution of the enveloped water to the net density of the colony. Only the cellular material contributes to the excess density of the entire colony since water is isopycnic with respect to itself. More surprisingly, Riegman *et al.* (1992) observed buoyancy (negative settling rate) of *C. socialis* colonies in one of their competition experiments. This means that the algae were able to decrease the net colony density (cells + enveloped 'water') below the density of the seawater. A comparable phenomenon has been documented for *Phaeocystis* colonies (see chapter 6). Cariou *et al.* (1994) observed that after a period of growth (≈ 4 days) on the bottom of unshaken culture vessels newly initiated *Phaeocystis* colonies began to float and continued their growth in the water column.

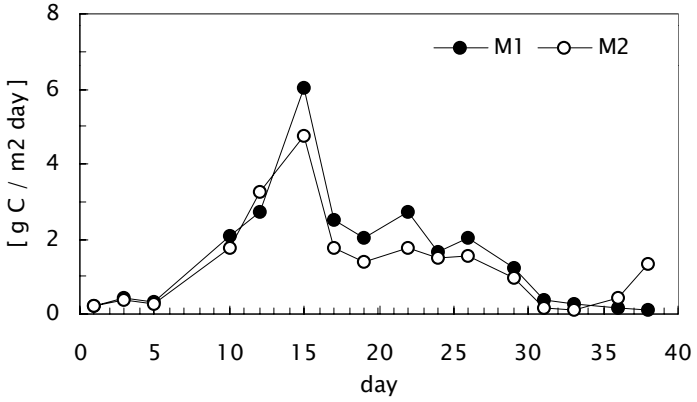

Figure 3.6. Daily primary production in mesocosm 1 (M1) and mesocosm 2 (M2).

The low turbulence in the mesocosms may have favoured *Phaeocystis* to the detriment of co-existing diatoms. Buoyant *Phaeocystis* colonies could remain in the well lit upper part of the water column (cf. Peperzak 1993) whereas diatoms, more evenly distributed over the water column, could experience less favourable light conditions. The high growth rate of *Phaeocystis* resulting from favourable light and non-limiting nutrient conditions may have led to an increase of biomass, diminishing the light available for diatom growth in the mesocosm.

Diatoms depend for their buoyancy on ionic pumps and the activity of these ionic pumps is strongly affected both by light and by the availability of respiratory energy (Anderson and Sweeney 1977, Anderson and Sweeney 1978). From an experimental study with *Ditylum brightwellii* Waite *et al.* (1992b) suggested that when light is unavailable, energy gained from respiration rate may be the principal determinant of sinking rate.

Van der Tol and Joordens (1993) calibrated a mathematical ecosystem model on the present experiment. The model pointed out that, with the available nutrients and light, diatom biomass should be higher than it was in the mesocosms after day 12. The only way for the model to compute the measured diatom biomass was to increase the sedimentation rate of the diatoms (but still within ecologically significant ranges). Fitting procedures can not be considered as evidence, nevertheless the model confirmed that the diatom decline was not induced by nutrients or light. An increased sedimentation rate is one of the possible processes which could have been responsible for the observed pattern.

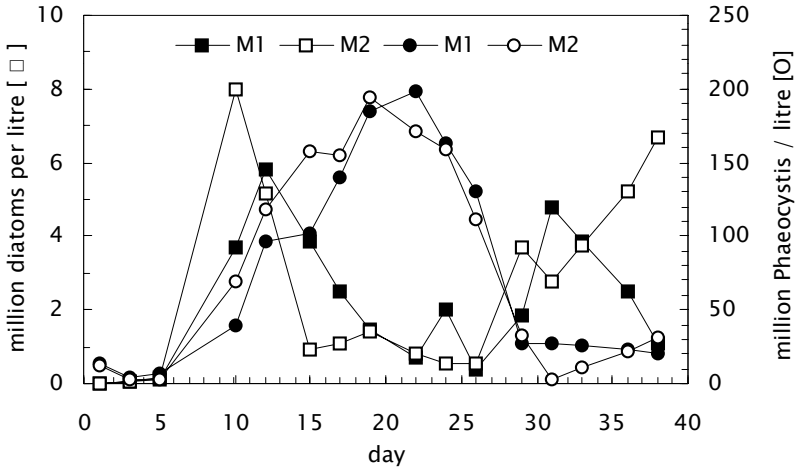

Figure 3.7. Diatom and *Phaeocystis* cell numbers in mesocosm 1 (M1) and mesocosm 2 (M2). (note different vertical scales)

selective grazing

Diatoms are considered to be a potential prey for many grazers whereas *Phaeocystis globosa* colonies are supposed to be less attractive food for microzooplankton and some mesozooplankton (Lancelot *et al.* 1987, Doering *et al.* 1989, Hansen and van Boekel 1991, Bautista *et al.* 1992, Fransz *et al.* 1992).
Selective grazing by copepods on diatoms may have been a reason for the decline of the diatom bloom in the mesocosms. Reduced copepod grazing pressure caused by the negative influence of a *Phaeocystis* colonial bloom on copepods, as suggested by Bautista *et al.* (1992) and Fernandez *et al.* (1992), may have allowed the reappearance of diatoms after the *Phaeocystis* colonial bloom. Specific data on zooplankton species composition and feeding during this mesocosm experiment are not available. However abundance of copepods was certainly low since they were never observed in the phytoplankton samples. This could result from the initial filtration of the mesocosm water over 100 µm gauze. *Oxyrrhis marina*, another potential phytoplankton grazer was also counted in both mesocosms during the experiment. Nevertheless it appeared only on day 24 and could not be responsible for the diatom decline taking place from day 12 onwards.

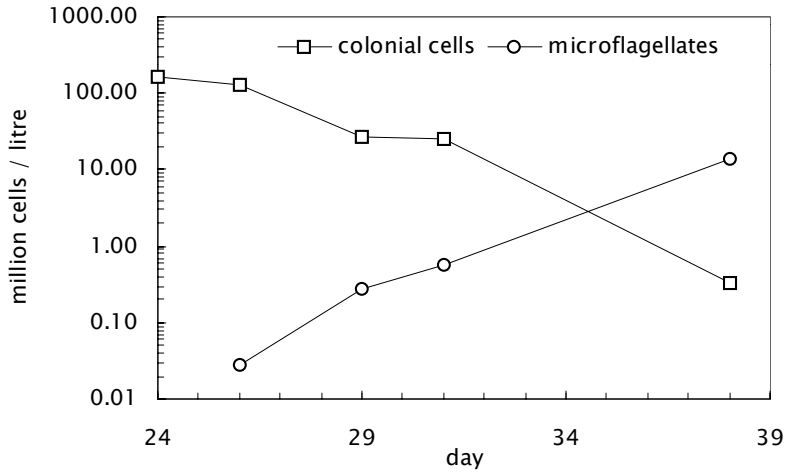

Figure 3.8. *Phaeocystis* colonial cells and solitary flagellates transition observed in mesocosm 1.

high temperatures

The relative high temperatures measured in the mesocosms could have favoured *Phaeocystis* to the detriment of the diatoms. During the growing phase of the diatoms (day 6 to 10), the temperature increased from 16°C to 20°C. Between days 12 and 25, i.e. the period when the diatom density decreased, the temperature remained in the same range. The decrease of the diatom density did not coincide with radical changes in the temperature, neither did the second diatom increase measured in the last week of the experiment. The temperature cannot be responsible for the observed species succession.

nutrient deficiency

Auxotrophic phytoplankton species are unable to synthesise some of their growth factors like vitamin B12 and they depend on the presence of these factors in the water (Bonin *et al.* 1981). This has been documented for centric diatom species e.g. *S. costatum* (Provasoli and Carlucci 1974, Swift 1980). If B12 binding factors are excreted in the water, as has been described for the haptophyte *Isochrysis* (Swift 1980), B12 is no longer available and the diatoms suffer from B12 deficiency. It could be hypothesised that the diatoms present in the mesocosm, e.g. *S. costatum*, were B12 auxotrophs and that *Phaeocystis* (also a haptophyte) produced B12 binding factors.

The wane of the *Phaeocystis* spring bloom in the North Sea has been attributed to nutrient depletion, either P (Veldhuis *et al.* 1986) or N (Lancelot and Mathot 1987, van Boekel *et al.* 1992). Van Boekel *et al.* (1992) found that a *Phaeocystis* colonial bloom declined through cell lysis induced by N limitation. Moreover nutrient depletion is said to be the factor causing ageing colonies to release motile single cells (Veldhuis *et al.* 1986, Verity *et al.* 1988b, Riegman 1991, Davies *et al.* 1992). Whereas Veldhuis and Admiraal (1987) observed colony formation at P concentrations below 0.2 μM, they also noted that the growth rate of the colonial cells was limited by P concentrations below 2 μM with an apparent half saturation constant for growth of 0.7 μM. Peperzak (1993) observed that, under nutrient-replete conditions, *Phaeocystis* cells remained at the solitary flagellate stage when the daily irradiance was below 100 W h m^{-2} day^{-1} and that colonies sinking below 100 W h m^{-2} day^{-1} released small flagellates.

Between day 15 and 17 P concentrations in both mesocosms were below 2 μM and mean water column daily irradiance decreased from 280 to 110 W h m^{-2} day^{-1}. During this period primary production, mainly attributed to *Phaeocystis*, began to decrease. We hypothesise that the drop in daily irradiance decreased the photosynthetic activity, thus reducing the energy available for P uptake and for buoyancy control (cf. Waite *et al.* 1992b). The simultaneous nutrient and light energy stress resulted in decreased buoyancy regulation, causing the colonies to sink and reach regions of even lower irradiance. This triggered the transformation of colonial cells into microflagellates (Peperzak 1993), as is illustrated by the observed appearance of ghost colonies accompanied by an increase of *Phaeocystis* microflagellates in the mesocosms. Moreover the colony destruction processes could have been intensified by increasing numbers and activity of bacteria measured in the same period (cf. van Boekel *et al.* 1992, Prins *et al.* 1993). The subsequent increase in light intensity and P concentration did not stimulate a resumption of the colonial bloom. Veldhuis and Admiraal (1987) observed that in cultures started with solitary cells the first colonies appeared after 7 days. In the present experiment the microflagellates appeared from day 26 onwards. The experiment ended on day 38. So no information is available about the further evolution of this flagellate population and about a possible delayed resumption of the colonial bloom.

Conclusions

A similar phytoplankton succession was observed simultaneously in both mesocosms. Contrary to the observations of Officer and Ryther (1980) and Egge and Aksnes (1992), *Phaeocystis* outcompeted diatoms in the presence of high Si concentrations. Relatively low turbulence and competition for light could also have been factors promoting the *Phaeocystis* dominance in the mesocosms. Moreover, selective grazing by zooplankton on diatoms, and vitamin deficiency of

diatoms caused by the presence of binding factors may also have played a role. However none of these hypotheses could until now be sustained with decisive evidence. The wane of the *Phaeocystis* colonial bloom to the profit of the flagellate form appeared to be triggered by transitory alterations of the environmental conditions: lowered irradiance combined with low P concentrations. These observations are in agreement with the findings of Peperzak (1993), who considered the transition of colonial to solitary cells to be part of the *Phaeocystis* life cycle, and an adaptive response to nutrient limitation.

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